REMARKS

I. Substance of Interview

Applicant is grateful for Examiner Singh's willingness to discuss the salient issues with applicant's representative during an interview conducted on November 17, 2011. As indicated in the examiner's Interview Summary Record, mailed December 8, 2011, the discussion focused on certain claim revisions, which applicant has endeavored to implement presently.

Applicant respectfully requests reconsideration of this application in light of the November 17th interview, the foregoing amendments, and these remarks.

II. Claim Status in View of Pending Action

Claim 8 is amended to recite the salient feature of claim 20, which is canceled presently without prejudice or disclaimer. Claim 8 is revised also to recite that the bispecific ligands are attached to the minicells and that the composition is free of membrane blebs of 200 nm or less in size, with support from the originally filed specification. At page 9, line 27, for instance, the specification discloses the attachment of bispecific ligand to minicells. Moreover, the specification provides that membrane blebs are removed from the minicell compositions with a 0.2 μm filter (see page 15, lines 18-22).

Claim 31 is amended likewise and claim 32 is canceled.

New claims 37-40 are added. Support for the new claims can be found, *e.g.*, in Example 10 and Figure 6, which demonstrate the therapeutic effects of 10^7 minicells loaded with 8.5 ng (G4) and 66 ng (G5) of a chemotherapeutic agent, doxorubicin. (See also discussion in Section III.B, *infra*.)

These changes thus introduce no impermissible new matter, warranting entry of the amendment. Upon such entry, which is requested, claims 8, 10-15, 17-18, 21-22 and 39-40 will be pending and under examination. Claims 31 and 33-38 are withdrawn from consideration.

III. Rejection Under § 103

The pending claims stand newly rejected over a combination of teachings that Examiner Singh has drawn from U.S. patent No. 7.183,105 (Sabbadini), Khatchatourians *et al.* (1973),

Christen *et al.* (1983), and Nikaido *et al.* (2003), among others. Yet the examiner acknowledges that, "[s]hould the claims be amended to limit any small molecule to ***chemotherapeutic drug*** and composition ***free of bacterial blebs of 200nm or less in size***, the above obviousness rejection may be overcome pending further considerations." Action at page 13, second paragraph (emphasis added). Without acquiescing the propriety of the obviousness rejection, applicant has amended claim 8 to comport with the examiner's comments.

Withdrawal of this rejection is warranted in light of the foregoing and applicant's previous response, the arguments of which are incorporated here by reference. Still, with respect to the cited prior art applicant would address as well certain points advanced in the action.

The examiner reads Nikaido in view of Schulz, *Curr. Opin. Cell Biol.* 5: 701 (1993), as teaching "a fast one-dimensional diffusion process" for loading a small molecule into a bacterial cell. Action, paragraph bridging pages 11 and 12 (emphasis in original). Such a reading of the prior art does not account adequately, however, for the relevant context for this Section 103 analysis; namely, that of a drug delivery method. A diffusion process also could not have achieved a therapeutically significant concentration for a chemotherapeutic agent or other small molecule drug, as presently recited. In particular, a "one-dimensional" diffusion mechanism, even as the examiner has interpreted Nikaido-*cum*-Schulz to implicate, could not have effected the loading into an intact, bacterially derived minicell of a small molecule drug, including both hydrophilic and hydrophobic chemotherapeutic agents, in a therapeutically significant concentration (compare claim 8).

A. Teachings by Nikaido-*cum*–Schulz of a simple diffusion process involving porin channels would not have lead the skilled artisan to the modified drug delivery method posited by the examiner

The examiner and applicant appear in agreement that Sabbadini does not teach the loading of a therapeutically significant concentration of a small molecule drug, *per se*, into bacterially derived, intact minicells. Thus, the examiner relies on the primary reference for "a ***method of delivery*** of small molecule[s] by attaching an antibody to a bacterial minicell[] that specifically binds a ligand present on the surface of a mammalian cell" (action at page 6, last full sentence). For an *a priori* predictable modification of such delivery method, on the other hand,

the examiner invokes the secondary references, primarily Nikaido read with Schulz, *supra*,[1] for evidence of the skilled artisan's reasonable expectation of adapting Sabbadini's method to "encapsulat[ing] … therapeutic[ally] effective concentration of small molecule drug in minicell[s] of approximately 400 nm … via a concentration gradient with entry via nonspecific porin channels in the outer membrane " (*id.*).

From the outset, applicant has emphasized that it was the discovery of the present inventors and not the prior art that intact, bacterially derived minicells can be loaded with "therapeutically significant concentrations" of small molecule drugs (specification at page 10, lines 21-23) and that the loaded drugs do not "move out of the minicells" after the loading (*id.* at page 24, lines 22 and 23). Furthermore, without acquiescing on the examiner's present reliance on Sabbadini, applicant submits that one of ordinary skill would have garnered exactly the opposite understanding from Nikaido.

That is, with respect to the "outer membrane (OM)" of Gram-negative bacteria, Nikaido would have informed the skilled artisan that "[d]iffusion" occurs "through the nonspecific porin channels," which "allow[] the influx of nutrients and perhaps … the extrusion of waste products" (page 594, line 2 of left column – line 6 of first full paragraph, right column). By the same token, Nikaido would have lent the understanding that a solute diffusing into a bacterial cell through such "nonspecific channels" down a concentration gradient, as proposed by Examiner Singh, would just as readily diffuse out again, once the gradient were reversed (see also discussion in next section). For the skilled artisan, moreover, such reversal would have seemed inevitable in relation to a method of delivery, *e.g.*, during various steps of carrier preparation, including purification, and ultimately upon systemic administration.

In relation to a method of delivery, therefore, Nikaido actually would have taught away from endeavoring to diffuse a chemotherapeutic agent or other small molecule drug through the OM of intact, bacterially derived minicells, as presently recited, because the skilled artisan would have expected, based on Nikaido, that the drug would not remain in the minicells upon the eventual reversal of the concentration gradient. Apparently recognizing this point, Examiner Singh has taken note of a theory, put forward by Schulz, that "revealed charged residues within

[1] Schulz (1993) is cited in the fifth line on page 12 of the present action.

[porin] channels result[] in a transversal electric field that separates polar and nonpolar solutes" (action, sentence bridging pages 11 and 12).[2] Elaborating on this theory, the examiner notes from Schulz "that polar solutes are thought to be oriented in the field during permeation," which the examiner contends causes "a fast one dimensional diffusion process" to arise as "an expected result [and] … the goal behind encapsulating an agent in the bacterial cell without any leakage or efflux" (*id.* at page 12, third full sentence; underscoring added).

In this last supposition, however, the examiner exceeds what the skilled artisan could reasonably have inferred in principle.[3] Instead, the person of ordinary skill would have understood Schulz's rumination about "a fast one-dimensional diffusion process" in the context of the author's focus on porin structure / function relations generally and his particular suggestion that a "transversal field is probably a general porin property" (page 704, last sentence of first full paragraph, right column). Thus, the skilled artisan would have recognized that Schulz had progressed from this suggestion to theorizing that the process of "permeation" through a porin channel by a "polar solute" could be likened to a planar or "one-dimensional" diffusion process, *i.e.*, one where the absence of net lateral solute movement, by virtue of the "orient[ing] in the field" of solute molecules, permits a relatively straightforward application of Fick's first law of diffusion.[4] Moreover, since reducing the dimensionality of diffusion-based processes was known to increase greatly the efficiency of biomolecular interactions,[5] the skilled artisan would have apprehended why Schulz qualified the theorized process as a "fast" one.

Under no circumstance, however, would one of ordinary skill have gleaned from Schulz or Nikaido an intimation, even in theory, that solute movement through bacterial porin channels

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[2] "It has been suggested that the electric field" thought to transverse a porin channel "separates polar and non-polar solutes. A polar solute … will be oriented in the field and will stay oriented during permeation, which therefore becomes a fast one-dimensional diffusion process." Schulz (1993), citing his own theoretical treatment in MEMBRANE PROTEINS: STRUCTURES, INTERACTIONS AND MODELS (Kluwer, 1992), pages 403-12.

[3] It also bears mentioning that even this overextension of the prior art would not have presaged applicant's demonstration, noted in the last response, that the claimed invention accommodates the loading into intact, bacterially derived minicells of a diversity of chemotherapeutic drugs and not just the polar solutes that, per the examiner's reading of Schultz, one would expect could move into a bacterial cell via porin channels. Thus, it is wholly unexpected that the claimed methodology can handle "a range of structurally dissimilar hydrophilic, hydrophobic and amphipatic drugs" (specification at page 10, lines 12-14).

[4] For example, see http://en.wikipedia.org/wiki/Fick's_laws_of_diffusion (visited February 15, 2012).

[5] *See* Gorman and Greene, *Nature Structural & Molecular Biology* 8: 768-74 (2008), citing Adam and Delbrück, "Reduction of dimensionality in biological diffusion processes," *in* STRUCTURAL CHEMISTRY AND MOLECULAR BIOLOGY (W.H. Freeman and Co., 1968) at pages 198-215.

would be *unidirectional*, as the examiner has intuited. Whether or not such movement is one-dimensional, per Schulz's suggestion, it most certainly occurs via a *diffusion* process, which necessarily is non-directional. Indeed, this is confirmed by Schulz's tacit invocation of Fick's first law, which presumes that diffusive flux goes from regions of high concentration to regions of low concentration, with a magnitude proportion to the concentration gradient (see text at footnote 3). Accordingly, the cited prior art would have impressed on the skilled artisan that solute entering into the cytoplasm of a bacterial cell via a porin channel, in a manner possibly treated as a fast one-dimensional diffusion process, would come out of the cytoplasm and into the extracellular environment as soon as a reversed concentrations gradient provided the requisite driving force.

B. <u>A therapeutically significant concentration of a chemotherapeutic agent in an intact minicell could not have been achieved by a diffusion process</u>

As noted above, the examiner finds in Nikaido a "clear[] teach[ing] that drug loading in [a] bacterial cell is likely by diffusion down a concentration gradient with entry [of such drug] via nonspecific porin channels in the outer membrane" of the bacterial cell (action at page 11, penultimate full sentence). Likewise, the skilled artisan also would have understood Nikaido to discuss the movement of a solute down a concentration gradient that existed between the outside and the inside of the bacterial cell, in keeping with the conventional definition of "diffusion" as the spread through random motion "of atoms or molecules from an area of higher concentration to an area of lower concentration." THE AMERICAN HERITAGE SCIENCE DICTIONARY, Houghton Mifflin Co. (2005).

In a sharply contrasting light, however, Example 10 and Figure 6 of the present specification, *inter alia*, illustrate applicant's claimed invention. More specifically, the example documents that 660 ng of doxorubicin in 10^8 minicells (6.6×10^{-6} ng/cell) proved therapeutically effective, whereas the efficacy of 0.85 ng doxorubicin in 10^6 minicells (8.5×10^{-7} ng/cell) was lower. In keeping with another of the inventors' discoveries, the present claims state that intact, bacterially derived minicells are about 400 nm in diameter. This means that the inner volume of an individual minicell is about 3.35×10^{-14} mL. Consequently, the aforementioned 8.5×10^{-7} ng/cell loading corresponds to a drug concentration of about 25 mg/mL, while the 6.6×10^{-6} ng/cell loading corresponds to about 197 mg/mL. Yet, the solubility of doxorubicin is only about

10 mg/mL. For instance, see www.inspiralis.com/go/anti_cancer_agents.php (visited February 14, 2012).

Accordingly, the simple diffusion process for which the examiner cites Nikaido-*cum*-Schulz could not possibly have achieved a drug amount reaching even threshold effectiveness, as applicant's example demonstrates for the amount of 8.5×10^{-7} ng per minicell (see also claim 37). Indeed, the recited "loading" of a chemotherapeutic agent manifestly is not a diffusing of the drug at all, as the previous section demonstrates.

This is evident, too, from Figure 1 of the application, which shows that incubation with 250 μg/mL doxorubicin resulted in the loading of 4.8 μg into 5×10^8 minicells. By calculations along the lines enumerated above, this translates into a per-minicell concentration of 286,000 μg/mL. In other words, the <*inside minicell / outside minicell*> drug concentration ratio is about 1000:1, or several orders of magnitude higher than the theoretical limit inferable for a simple diffusion process.[6]

[6] This aspect of the invention is all the more surprising given the fact, noted in applicant's last response, that "bacterial cytoplasm (and, hence, minicell cytoplasm) contains significant concentrations of biocompatible solutes. Thus, it was believed that there might be insufficient spare intracellular space to accommodate high concentrations of non-biocompatible drug solutes, without loss of minicell integrity" (specification at page 10, lines 21-27). Furthermore, Nikaido states that bacterial such as *E. coli* "build membrane barriers that … prevent the permeation of … noxious solute molecules, and that "toxic molecules that slowly leak in through these membranes are actively pumped out" (page 629, right column). Accordingly, the inventors' discovery that minicells do not keep our or expel chemotherapeutic agents as noxious solutes (see specification, *e.g.*, at page 10, lines 15 *ff.*) contradicts the examiner's basic generalization from bacterial cells (Nikaido, Schulz, etc.) to intact, bacterially derived minicells.

CONCLUSION

In view of the foregoing, applicant submits that the pending Section 103 rejection is without merit and should be withdrawn. The non-obviousness of applicant's claimed invention is substantiated as well by the published, third-party acknowledgements of its surprising character (see last response at page 11, last paragraph, and accompanying supplemental IDS).

Applicant further submits, therefore, that the application is in condition for allowance, and an indication to this effect is requested. Examiner Singh also is invited to contact the undersigned directly, should she feel that any issue warrants further consideration.

Respectfully submitted,

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/Stephen A. Bent/
Stephen A. Bent, Reg. No. 29768
Attorney for Applicants

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5404
Facsimile: (202) 672-5399

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